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A Study of Some Erythrocyte Indices and Bacteriological Analysis of Broiler-chickens Raised on Maggot-meal Based Diets

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Abstract: Five treatment groups of 18 broiler-chickens each from age 4-9 weeks were fed diets in which maggot meal (MGM) replaced 0, 25, 50, 75 and 100% of fish meal (FM) on equi-protein basis. Ethyledine Di Tetra Acetic acid (EDTA)-treated blood samples were taken from each of the groups and subjected to total erythrocyte counts, packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV). Results therefrom showed no significant ($p > 0.05$) difference and also compared favourably with the normal physiological values for these indices in healthy chickens. Isolation and characterization of bacterial organisms in the different broiler-chicken diets formulated with varying levels of maggot meal obtained from fresh droppings of the experimental chickens and some visceral organs in addition to development medium of the maggot were carried out. Findings revealed that most bacteria associated with the broiler-chickens fed maggot meal-based diets in this study are by far those that are commonly experienced by man and animals in their day to day exposure and to which their bodies have developed some relative immunity. It was concluded that since MGM inclusion in the diets of broiler-chickens does not negatively influence the erythrocyte indices and that the bacterial flora isolated from the different parts of the experimental chickens were similar to those found in fish meal fed diets, MGM could effectively replace the more expensive FM without threatening the blood physiology of the chickens and health of consumers of such broiler-chickens.

Key words: Maggot meal, visceral organs, Ethyledine Di Tetra Acetic Acid

Introduction

Broiler production is a high-risk sensitive enterprise which is full of challenges of nutrition, health and management. The importance of feed in the venture is evidenced by a proportion of 75-80% of the production cost (Fetuga, 1977; Eckman, 1995). Protein, which is the most expensive portion of the feed (Fetuga, 1977) is conventionally sourced from groundnut cake (GNC), soya-bean meal and fish meal which are keenly competed for by man. The search for the least-cost formulations is currently exploring the replacement of these expensive feed raw materials with cheaper alternatives in the formulation of poultry ration. Appropriate strategy for this includes the use of locally available new ingredients (Aletor, 1986; Kita and Okumara, 1993). Such inexpensive raw materials that have been found to replace fishmeal in the broiler-chicken diets include earthworm (Fosgate and Babb, 1972), maggots (Teotia and Miller, 1974; Atteh and Ologbenla, 1993) and industrial by products (Aletor, 1986).

Although the use of unconventional feed-sources such as MGM reduces the competition between man and livestock for conventional feed raw materials, information is scanty on their effects with regards to the health and performance of the chickens and their consumers. Also, in spite of it's ready availability, the public acceptability of MGM as chicken feed ingredient may be in doubt because of the concerns of chicken consumers as maggots have traditionally been associated with decaying organic matters.

Public attention has been drawn to the health implication of including maggot meal in livestock diet because it develops in decaying and filthy products (Calvert *et al*.,1969; Atteh and Adedoyin, 1993) and because the adult form the mature housefly (*Musca domestica*), disseminates disease causing organisms (Matanmi, 1990). *Escherichia coli* infection which is responsible for major losses in the poultry industry, is commonly found in poultry litter and faecal materials. Bio-deterioration takes place in poultry manure as part of the metabolic activities of the bacteria present in it (Onion *et al*.,1981) and some of these micro-organisms are pathogenic and are harboured by the larvae which develop in the manure.

The physiological importance of erythrocytes in the domestic livestock has prompted studies that led to the establishment of some indices with which the health and performance of the animals can be monitored. More important among such health conditions is anaemia which can be monitored using indices such as packed cell volume (PCV),Total red blood cell (RBC) count, Haemoglobin (Hb) content, Mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC). As part of the objectives of this study, the effect of

maggot meal based diets on broiler-chicken erythrocyte indices was monitored as deviation from normal values may affect the physiological status of the chickens and consequently their performance.

Also, isolation and characterization of bacterial organisms in the different broiler-chicken diets formulated with varying levels of maggot meal as well as microbial loads in the liver, kidney and spleen of such birds raised to maturity on maggot-meal based diets was also investigated so as to determine the suitability of such chickens for human consumption as a way of safeguarding the health of the public.

Materials and Methods

Maggot Meal Production: Fresh maggots were extracted from the manure collected from the pit under the layer-chicken cage.

Homogeneously mixed pasty fresh poultry manure was introduced into wooden boxes of same surface area ($0.2025m^2$) but different depths of 0.05m, 0.1m, 0.15m and 0.2m. The manure in each box was regularly moistened by water sprinkling to ensure wetness, fly attraction and oviposition. The setup was observed daily till the first pupa appeared (indicating that maggots were due for harvesting). The developed maggots were harvested using the floatation technique whereby a hand-bowl of manure impregnated with maggot was introduced on a plastic mesh (sieve) immersed in a basin of water. The manure was dissolved by gentle manual vibration of the sieve in water and washed off leaving clean maggots. The maggots collected were processed by sun or oven-drying between 37-41^{o}C for about 96 hours after which they were milled.

Feed Composition: The milled MGM was mixed with other feed ingredients at varied proportions as indicated in Table 1.

Experimental chickens: One hundred and two (102) day old Anak broiler-chicks were procured from a commercial farm. The chicks were brooded under standard conditions at the university's Teaching and Research Farm. They were fed 24% protein formulated broiler diet for a pre-experimental period of three weeks during which they were vaccinated at intervals against some endemic viral infections such as Newcastle disease (ND), Infectious bursal disease (IBD) and Fowl pox disease.

Feeding Trial: On the first day of the fourth week, 90 of the birds were removed from the broiler unit and divided into 5 treatment groups of 18 chicks per group such that the mean group weights were identical. The chicks were thereafter randomly assigned to treatment diets formulated with maggot meal (MGM) replacing 0, 25, 50, 75 and 100% fish meal (FM) on equi-protein basis. Each treatment was replicated thrice. All diets were made iso-nitrogenous containing approximately 21.76±0.15% crude protein with FM level of inclusion being limited to 4% for cost effectiveness. The broiler chickens were fed *ad libitum* until they were nine weeks when haematological studies were carried out.

Haematological Studies: On the first day of the tenth week, the birds were deprived of feed overnight and two chickens (a male and a female) were randomly selected from each replicate totaling six chickens per treatment. Blood samples were collected from each of the birds by jugular venipuncture into well labeled sterile universal bottles (one set containing a pinch of anticoagulant powder (EDTA) while another set of bottles did not. These samples were used in determining the set parameters according to the methods described by Schalm *et al.*, 1975; Oyewale, 1987. The mean values obtained for the determination of the various indices in respect of the 5 treatment groups were subjected to Analysis of Variance (ANOVA).

Identification and characterization of Bacteria

Isolation of Bacteria: One gram (1 gm) of each sample was put into MacCartney bottle containing 5ml of sterile saline solution (85%) and mixed thoroughly. Serial dilutions were obtained from this up to 10^{-4}; 1ml of each mixture was transferred into molten nutrient agar (20ml) plates and incubated at 37, 42 and 47^{o}C for 24 hours. Individual colonies from each plate was sub-cultured until pure cultures were obtained.

Characterization of Bacteria: Bacteria were identified using the following criteria: morphology, Gram staining, catalase test, motility, oxidase test, oxidation-fermentation and spore staining. The modified methods of Cowan and Steel (1974) were used.

Bacterial Count: After 24 hours of incubation, the colonies on the medium were counted using the electronic colony counter. Six out of the 54 squares on the counting machine were randomly selected and counted .The mean of the two counts for each treatment was determined and the value obtained was multiplied by 54 squares to obtain the population density of bacteria (cfu/g) in each culture of the visceral organs and faecal collections.

Results

Erythrocyte Indices: The erythrocyte values obtained for each experimental diet in this investigation are presented in Table 1. The other erythrocyte indices such as PCV, MCHC, MCV and Hb were within the same range as obtained by Mitruka and Rawnsley (1977); Ross *et al.* (1978).

Table 1: Composition of Experimental Diets (g/100g)

Raw Materials	Diets				
	1	2	3	4	5
	% FM protein replaced by MGM protein				
	0	25	50	75	100
Maize	57.00	56.83	56.66	56.47	56.32
Groundnut cake (GNC) 45.1%	28.00	28.00	28.00	28.00	28.00
Fish meal (FM) 64.5%	4.00	3.00	2.00	1.00	0.00
Maggot meal (MGM) 55.1%	0.00	1.17	2.34	3.51	4.68
Brewer's dried grain (BDG)	5.00	5.00	5.00	5.00	5.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Vitamin/Mineral Premix	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Calculated analysis	100.00	100.00	100.00	100.00	100.00
Crude protein (g/100g)	21.78	21.76	21.75	21.73	21.71
ME (Meal kg+)	3.09	3.09	3.09	3.09	3.09
Methionine + Cystine	1.08	1.14	1.04	1.11	1.16
Lysine	3.00	2.95	2.55	2.92	2.92

Table 2: Erythrocyte Indices obtained from Broiler-Chickens Fed Different Levels of MGM

Diet	% FM protein replaced by MGM	RBC Count (g/100cm^3)	Haemoglobin (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (UM^3)
1	0	2.3 ± 0.2	7.9 ± 0.1	29.7 ± 1.3	27.3 ± 1.1	34.6 ± 2.4	125.6 ± 11.8
2	25	2.7 ± 0.8	7.7 ± 0.2	34 ± 3.8	23.6 ± 0.6	29.00 ± 2.2	126.7 ± 13.3
3	50	2.2 ± 0.2	7.5 ± 0.1	31.8 ± 0.6	26.2 ± 2.5	35.00 ± 3.5	129.1 ± 11.5
4	75	2.7 ± 0.2	8.0 ± 0.4	32.2 ± 3.8	26.2 ± 2.5	32.00 ± 1.0	124.5 ± 18.5
5	100	2.3 ± 0.1	7.5 ± 0.9	29.6 ± 1.0	25.9 ± 0.6	32.6 ± 1.7	126.9 ± 8.2

Bacterial isolates and their characterization: The characteristics of bacterial isolates in the diet, visceral organs and faecal collections from the experimental chickens are given in Table 3.

Uniquely, it was observed that no bacterial organism was isolated in diet 5 which has 100% FM replaced by MGM at the birds' core temperature of 41-42°C.

Bacterial isolates in the culture of maggot washing incubated at different temperature regimes included *Bacillus* species, *Salmonella* sp and *Corynebacterium* sp at 37°C; *Lactobacillus* sp and *Staphylococcus* sp at 42°C while *Bacillus* sp, *Clostridia* sp and *Escherichia* sp were isolated at 41°C.

The population count (colony forming units/ml) for the bacteria culture of the visceral organs and faecal collections of broiler chickens fed with various maggot meal-based diets as observed in the diets was in the order Diet 1 Diet 4 Diet 3 Diet 2 Diet 5. (Table 4).

Discussion

The results obtained from this study suggest that the feeding of maggot meal to broiler chickens has no adverse effect on erythrocyte indices because all the values for PCV, Hb, MCHC and MCV obtained in this study compared favourably well with normal values as obtained by Mitruka ans Rawnsley (1977) and Ross *et al.* (1978) as indicated in Table 2. The findings that none of the broiler-chickens' died during the 6-week feeding trial and that the values obtained for the erythrocyte indices for all the experimental diets were insignificant are reliable indications that maggot meal based diets are not inferior to fish meal as reported by Awoniyi and Aletor (1999).

The various differences in the culture temperature observed for various bacteria in the study corroborated the findings of earlier studies (Olutiola *et al.*, 1991) which led to the classification of bacteria on the basis of temperature into Psychrophiles, Mesophiles and *Thermophiles*. The same reason probably accounts for why some organisms do not survive inside the birds when consumed in diets. The high core temperature of the birds confers a natural infection control device in the birds against some bacteria.

Table 3: Bacterial organisms isolated in the visceral organs and diets formulated with graded maggot meal (MGM)

	Visceral organs						Diets				
Micro organisms	H	S	L	K	LV	P	1	2	3	4	5
Bacillus	+	+			+	+	+	-	+	+	+
Staphyloccus	+	+	+	+	+	+	+	+	+	-	+
Micrococcus	+	+	+	+	+	+	+	+	+	+	
E. coli							+	+	+	+	+
Pseudomonas							-	+	+		
Clostridia							+	-	-	+	+
Yersinia							+	+	+	+	+
Corynebacteria									+	+	+

H = Heart, S = Spleen, L = Lungs. K = Kidney. LV = Liver. P = Pancreas

Table 4: Population count (colony units per ml) for the Bacteria culture from the visceral organs and faeces of broiler chickens fed different MGM diets

	Visceral organs		Faeces	
Diet	Sum	Mean ± SD	Sum	Mean ± SD
1	10.1×10^5	$5.05 \pm 0.25 \times 10^5$	10.0×10^3	$5.0 \pm 1.4 \times 10^3$
2	5.12×10^5	$2.56 \pm 0.08 \times 10^5$	2.86×10^3	$1.43 \pm 0.4 \times 10^3$
3	5.94×10^5	$2.97 \pm 0.72 \times 10^5$	3.66×10^3	$1.83 \pm 0.6 \times 10^3$
4	7.51×10^5	$3.75 \pm 0.93 \times 10^5$	3.01×10^3	$3.01 \pm 0.8 \times 10^3$
5	1.01×10^5	$0.51 \pm 0.15 \times 10^5$	14.38×10^3	$7.19 \pm 1.1 \times 10^3$

The reason for the occurrence of nearly similar bacterial organism characterized in the cultured organs of birds fed different maggot meal based diets may be related to the fact that all the diets were mixed with maggot meal from the same source (that is in bulk at the same time and from the same environment). Consequently, maggots required for livestock feed inclusion should be sourced from a stock of flies that are disease free .

The population count for bacteria in culture of exercised organs showed that organs excised from the broiler chickens fed Diet 5 which had 100% FM replaced with MGM and expected to have undergone greater bio-degeneration (bacterial activity) had the least bacteria population count whereas Diet 1 (100%FM) had a higher count.

From the population count study, the total bacterial count observed in the visceral organs of birds fed Diet 1 ($5.05X10^5$) colonies units per ml when compared to the counts for the visceral for birds on diets with values $2.56X\ 10^5$, $2.97X10^5$, $3.75X10^5$ and $0.51\ X\ 10^5$ for diets 2, 3, 4 and 5 respectively suggests that the maggot meal inclusion has no serious bacterial threat than the handling and management of the feed during formulation to ensure a hygienic procedure.

However ,it must be emphasized that such factors as poor hygiene, deteriorative changes in the ration (poor keeping quality and the immune status of the chickens may influence the population of the microbes thus potentiating their pathogenicity.

From earlier reports by Shapiro and Sarles (1949); Smibert *et al.* (1958); Fuller (1973); Thornton and Gracey (1976); Bains (1979), it could be concluded that most of the bacteria occurring in the broiler chickens fed maggot-meal based diets in this study are by far those that are commonly experienced by man and animals in their day to day exposure and to which their bodies have developed some degree of relative resistance.

The study clearly indicates that maggot meal can completely replace fishmeal in broiler-chickens diet without adverse effects on their erythrocyte indices. Consequently, more research should be focused on commercial production of MGM to meet the massive industrial requirements of the Nigerian poultry population.

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